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Salmonellosis in Finishing Pigs in Spain: Prevalence, Antimicrobial Agent Susceptibilities, and Risk Factor Analysis

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ABSTRACT

A herd-based survey of *Salmonella* in pigs was carried in a major pig producing region of Spain. Mesenteric lymph nodes were collected from the carcasses of 25 pigs from each of 80 herds at time of slaughter. *Salmonella* spp. were isolated from 31% of animals and 94% of herds. Within-herd prevalence ranged from 4 to 88%, with the prevalence in most herds being greater than 10%. A large diversity of *Salmonella* serotypes was found, with Typhimurium, 4,[5],12:i:−, and Rissen being the most prevalent. Two or more serotypes coexisted in 73% of the herds. *Salmonella* Typhimurium was present in 68% of the herds. Most (82%) of the *Salmonella* isolates belonged to serogroups targeted by enzyme-linked immunosorbent assay tests for pig salmonellosis. Resistance to at least one antimicrobial agent was detected in 73% of the strains, and one or more resistant strains were recovered from pigs in 93% of the herds. Antimicrobial agent resistance (AR) was more frequent among the most prevalent than it was among the rarer serotypes. Twenty-five multi-AR patterns were found. Resistance to three or more families of antimicrobial agents was found in 75% of AR strains. The finding that many of the herds yielded isolates of several multi-AR patterns indicates that *Salmonella* infections were acquired from multiple sources. High prevalence of *Salmonella* in herds was associated with lack of rodent control programs, herds from farms with only finishing pigs, herds managed by more than one full-time worker, herds for which the source of drinking water was not a city supply, and relatively long fattening times.

Salmonella spp. are recognized as major zoonotic pathogens of economic significance in animals and humans; the infection caused by these bacteria is one of the most frequently reported foodborne diseases worldwide and the second most important zoonosis in the European Union (EU) (after campylobacteriosis) *(11)*. In 2008, 131,468 cases of human salmonellosis were registered in the EU, 3,833 of which occurred in Spain.

In response to the need to protect consumer health, the EU initiated a process to monitor zoonoses and zoonotic agents, specifically, the control of *Salmonella* and other specified zoonotic agents transmitted by foods *(3)*. This regulation establishes the obligation of member states to set up specific control programs throughout the entire food chain. For this purpose, the EU recently commissioned a study to estimate the prevalence, identify major serotypes, and characterize epidemiology and antimicrobial agent resistance of *Salmonella* infection in slaughter and breeder pigs in each member state. Results from this study reveal a mean *Salmonella* prevalence in slaughter pigs of 10%, with a wide variation among member states (from 0% in Finland to 29% in Spain) *(7)*. Based on these prevalence results, the EU is now in the process of setting up country-specific targets for reducing *Salmonella* prevalence in swine. Because many factors could explain the large discrepancies in *Salmonella* prevalence among countries (i.e., bioclimatic characteristics, type of rearing and production systems, feeding practices, interlaboratory variability, etc.), the European Food Safety Authority (EFSA) also recommends initiating country-level studies aimed at gaining a better knowledge of the epidemiology of pig salmonellosis *(8)*. The different control measures implemented by each member state, based on their different political and/or economical decisions, might also play an important role on the prevalence of this infection. In addition, the number of *Salmonella* strains resistant to multiple antibiotics has increased considerably in recent years. Thus, up-to-date data on pig salmonellosis will help define the activities and control measures to be best implemented in each particular region.

Because almost 45% of the Spanish pig population is concentrated in the northeastern portion of the country (autonomous regions of Catalonia and Aragon), the epidemiological characterization of the *Salmonella* infection in this particular area should be considered crucial in order to establish economically feasible control programs in Spain.

Unlike the EU study based on bacteriology of mesenteric lymph nodes (MLN) from individual pigs, we carried out a herd-based survey. The main objectives of this study were to (i) evaluate the individual and the herd prevalence of *Salmonella* spp. and the most frequent serotypes in finishing pigs units in northeastern Spain, (ii)

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FIGURE 1. *Map of the region of Aragon, in northeastern Spain. Dots represent the location of the abattoirs included in the study.*

assess the presence of the *Salmonella* antibiotic-resistant (AR) strains in the region and the main AR profiles, and (iii) determine major factors associated to the presence of *Salmonella* infections in the studied pig population.

MATERIALS AND METHODS

Study design and sample collection. The sampling was carried out on six abattoirs (one in the Province of Huesca, three in Teruel, and two in Zaragoza), which represented approximately 75% of the slaughtered pigs in Aragón, northeastern Spain (Fig. 1). Two of them were large abattoirs, slaughtering more than 300 pigs per hour, two other abattoirs slaughtered between 150 and 300 pigs per hour, and the remaining two slaughtered fewer than 100 pigs per hour. The target population included all finishing pig farms within the region that submitted pigs to abattoirs located in the region.

Between February 2008 and December 2009, 1,997 pigs from 80 pig herds (an average of 25 animals per herd) were analyzed. On the day of sampling, 25 pigs from one of the producers delivering animals to the abattoir that day were selected in a random fashion from the slaughter line. A minimum of 25 g of MLN (from at least five nodes) was collected from each animal. Samples were processed within the first 24 h. The number of herds sampled in each abattoir was proportionally distributed to the slaughtering carried out by each abattoir in the region.

Herd data collection. Initially, a simple questionnaire was administered to the abattoir where the animals were slaughtered to collect data on (i) the farm location, (ii) the company name, (iii) the number of pigs in the batch, (iv) the time of arrival, and (v) the time of slaughter. A larger questionnaire was designed to obtain information regarding different aspects of the fattening farm to which slaughtered pigs belonged. An initial draft of the questionnaire was pretested to two swine practitioners to ensure that the questions were easily understood by the farmers. Suggested modifications were included in the final form. The questionnaire was divided into five main sections: (i) farm general characteristics such as type of farm (strict finishing versus farrow to finish), herd size, number of fattening units, percentage of slatted floor, separation between boxes (solid walls versus bars), box stocking density, length of the fattening period, number of full-time workers, etc.; (ii) farm biosecurity, i.e., proper maintenance and use of the outside fence and sanitizing foot and wheel baths, the wearing of specific clothes before entering the facilities, the presence of a changing room, restrictions on people visiting the farm, sharing employees with other farms, presence of other domestic animals, wild birds and rodents, rodent control programs, etc.; (iii) feeding management, i.e., automatic versus manual feeding, type of feed (pellets versus grain–mash), number of different diets during the fattening period, amount of feed offered (restricted versus ad libitum), water supply (city water, wells, irrigation channels, rivers), etc.; (iv) farmer's characteristics, namely, age, education (no studies, primary, secondary or university degree), and additional training on pig production; and (v) antimicrobial agent(s) use, i.e., antibiotic name, period of the fattening time within which it (they) were used, number of days of treatment, and purpose (preventive, growth promoters, or treatment of health problems). The questionnaire was administered to the farmers by way of their respective veterinarians, who were asked to mind the farmers' answers.

Determination of *Salmonella*. Bacteriology was performed on MLN, following the standard International Organization for Standardization Method 6579:2002 *(2),* as recommended *(5).* In short, MLN were removed from the intestinal package, free of fat or connective tissue and decontaminated before analysis by dipping into absolute alcohol and further flaming of the external surface. Twenty-five grams of externally decontaminated MLN was homogenized in a Stomacher blender (Seward Medical, Ltd., London, UK), placed in 225 ml of buffered peptone water, and incubated for 18 $\pm$ 2 h at 37 $\pm$ 1°C. Three drops (33 μl each) of incubated buffered peptone water were inoculated into modified semisolid Rappaport-Vassiliadis medium, and plates were incubated for 24 $\pm$ 3 h at 41.5 $\pm$ 1°C (negative samples were reincubated for an additional 24 h). One microliter of presumptive *Salmonella* growth from each sample (detected by the halo generated in modified semisolid Rappaport-Vassiliadis after 24 or 48 h) was transferred to two selective media (xylose lysine deoxycholate and brilliant green). Suspect colonies were confirmed biochemically (triple sugar iron agar, urea agar, L-lysine decarboxylation medium, and an indole reaction), and serotyping was performed at the National Center for Animal Salmonellosis (Madrid, Spain), following the Kaufmann-White scheme *(38).* Only one colony from each positive culture was picked for serotyping. *Salmonella* strains were stored at −20°C and further lyophilized in sterilized Bacto skimmed milk (Difco, BD, Sparks, MD) supplemented with 1% lactose (Lattosio, Carlo Erba, Italy).

Antimicrobial agent susceptibility testing. To ensure a representative selection of strains from the collection, at least one strain of each serotype isolated from each herd was randomly selected for further analysis of AR. When there was more than one

strain of a given serotype in a herd, two strains were randomly chosen. The selected *Salmonella* isolates were tested against a panel of 19 antimicrobial agents selected by (i) the current EU regulations for the harmonized vigilance of the *Salmonella* resistance in pig farms *(6)* (i.e., nalidixic acid, ciprofloxacin, cefotaxime, ampicillin, chloramphenicol, streptomycin, gentamicin, sulfisoxazole, trimethoprim, and tetracycline), and (ii) the nine agents used more frequently in swine farms, according to the results obtained from the questionnaires and expert (swine practitioners) opinion (i.e., doxycycline, colistin, amoxicillin, amoxicillin–clavulanic acid, enrofloxacin, sulfamethoxazole-trimethoprim, ceftiofur-spectinomycin, and neomycin). The antimicrobial agent susceptibility test was performed using the Kirby-Bauer disk diffusion method *(37)*. Antimicrobial agent concentrations used where those recommended by the European Committee on Antimicrobial Susceptibility Testing *(6)* and the Clinical and Laboratory Standards Institute (CLSI) *(4)*. In addition to the standard *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028 and ATCC DT104 reference strains were used as controls of each experiment. *Salmonella* susceptibility was determined by measuring the inhibition halo, and each strain classified as resistant, intermediate, or susceptible, according to CLSI guidelines *(4)*. Farms showing at least one *Salmonella* strain with a resistant or intermediate phenotype to at least one antimicrobial agent were classified as AR to that specific antibiotic.

For determining the main AR profiles, antimicrobial agents were classified by families, according to World Organization for Animal Health's (Office International des Épizooties [OIE]) ''List of Antimicrobials of Veterinary Importance'' *(10)*: first-generation quinolones (nalidixic acid), second-generation quinolones or fluoroquinolones (enroflaxin and ciprofloxacin), third-generation cephalosporins (cefotaxime and ceftiofur), aminopenicillins (ampicillin, amoxicillin, and amoxicillin–clavulanic acid), phenicols (chloramphenicol), aminoglycosides (streptomycin, spectinomycin, gentamycin, and neomycin), sulfonamides (sulfisoxazole, trimethoprim, sulfamethoxazole-trimethoprim), tetracyclines (tetracycline and doxycycline), and polypeptides (colistin).

Statistical analysis. Before any analysis, data were checked for inconsistencies, data entry mistakes, and missing values, and corrected when possible. Descriptive statistics were performed, and individual and herd prevalences with their corresponding 95% confidence intervals (95% CI) estimated. Given the specificity of bacteriology (100%), a herd was considered positive when *Salmonella* was isolated from at least one animal in the batch. Data were analyzed with STATA software (StataCorp, L.P., College Station, TX).

Farm-level information was used to assess further whether *Salmonella* prevalence was related to some of the variables characterizing the herd. Farm-level variables were defined as exposures to the individual farm animals that might influence microbiological results. Because animals were grouped by farm, a multivariable random-effect logistic regression was used, in which the outcome variable was being culture positive, variables from the questionnaire were the explanatory variables included in the model as fixed effect, and the herd was included as the random effect. Because of the large number of variables collected, univariable random-effect logistic regression analysis assessing the relationship between each factor and the outcome variable were performed first as a screening step. Variables with a significant relationship ($P < 0.1$) with infection were tested in the multivariable model. Continuous variables were categorized based on percentiles before analysis. The multivariable regression model was constructed with a stepwise approach, in which variables showing a P value ≤ 0.1 were entered into the model, and all that showed a P value ≤ 0.05 in the model were finally retained. Province of origin of the herd and abattoir were forced into the model as potential confounders, regardless their significance. Biologically plausible two-way interactions between variables in the model were assessed as well.

The relationship between the use of antibiotics and the presence of AR *Salmonella* strains in herds was assessed by chi-square analysis. *Salmonella* prevalence in herds presenting AR to a given antibiotic was also compared with prevalence in those classified susceptible to the same drug by using the Kruskal-Wallis test.

RESULTS

Descriptive data and serotypes. *Salmonella* spp. were isolated from 625 (31.3%) animals (95% CI = 29.1 to 33.5%). Seventy-five (93.7%) herds (95% CI = 88.7 to 98.8%) had at least one infected animal. The prevalence within the positive herds ranged from 4 to 88%, but most of infected pig farms (88%) showed more than 10% of animals infected.

Thirty-seven different serotypes belonging to 12 serogroups were identified (Table 1). Around 60% of the isolated strains belonged to three serotypes: *Salmonella* Typhimurium (37%), *Salmonella* Rissen (12.5%), and *Salmonella* 4,[5],12:i:− (12%). *Salmonella* Typhimurium was present in nearly 70% of the infected pig farms, being by far the serotype more widespread (Table 1). However, in 55 (73.3%) farms, two or more circulating serotypes of *Salmonella* were isolated. Six serogroups (B, C1, C2, D1, E1, and Y) represented 96.2% of the isolates, serogroups B and C1 being the most frequently found on farms (97.3%) and animals (97.5%). It is noteworthy that 17.6% of *Salmonella* isolates (present in 36% of the infected pig farms) belonged to serogroups antigenically unrelated to those usually detected by commercially available enzyme-linked immunosorbent assay tests (B, C1, and D1).

AR. Two-hundred seventy-eight *Salmonella* strains (44.5% of the isolates) belonging to 74 infected pig farms (one farm could not be analyzed) and to the 37 serotypes were analyzed against the 19 antimicrobial agents described above. AR to at least one antimicrobial agent was found in 73.4% of the strains (Table 1) and was present in 93.2% of the infected pig farms. Overall, the most prevalent serotypes, Typhimurium, Rissen, and 4,[5],12:i:−, showed a percentage of strains with AR significantly higher than those percentages of the other serotypes taken together (95.3 versus 47.6%; chi-square = 80.1; $P < 0.0001$). There were, however, four *Salmonella* serotypes less prevalent, Anatum, Kapemba, Wien, and London, which showed AR in all of the strains analyzed.

Of these 204 AR strains, the greater number was observed against tetracycline (86.8%), doxycycline (82.4%), sulfisoxazole (76.5%), streptomycin (72.1%), and amoxicillin or ampicillin (70%). No AR against fluoride quinolones (enroflaxin and ciprofloxacin) was detected. However, only two strains, 4,[5],12:i:− and Rissen, showed resistance to colistin, and two, Bredeney and Muenster, were resistant to third-generation cephalosporins (Table 2).

TABLE 1. *Individual and farm serotype prevalence and AR of* Salmonella *strains isolated in finishing pigs in northeastern Spain*

Serotype/subspecies	Serogroup	No. (%) of infected herds[a]	No. (%) of infected pigs[b]	No. (%) of strains analyzed for AR[c]	No. (%) of resistant strains
Typhimurium	B	51 (68.0)	231 (37.0)	86 (30.9)	81 (94.2)
4,[5],12:i:−	B	22 (29.3)	75 (12.0)	32 (11.5)	31 (96.9)
Rissen	C1	18 (24.0)	78 (12.5)	32 (11.5)	31 (96.9)
Derby	B	8 (10.7)	33 (5.3)	12 (4.3)	11 (91.7)
Mikawasima	C1	7 (9.3)	10 (1.6)	10 (3.6)	2 (20)
arizonae	Y	6 (8.0)	22 (3.5)	10 (3.6)	2 (20)
Newport	C2	5 (6.7)	12 (1.9)	8 (2.9)	0 (0)
Anatum	E1	4 (5.3)	13 (2.1)	6 (2.2)	6 (100)
Goldcoast	C2	4 (5.3)	8 (1.3)	6 (2.2)	5 (83.3)
Muenchen	C2	4 (5.3)	4 (0.6)	4 (1.4)	1 (25)
Bredeney	B	3 (4.0)	5 (0.8)	5 (1.8)	3 (60)
Kapemba	D1	3 (4.0)	11 (1.8)	5 (1.8)	5 (100)
Enteritidis	D1	3 (4.0)	10 (1.6)	5 (1.8)	2 (40)
Give	E1	3 (4.0)	9 (1.4)	5 (1.8)	0 (0)
Reading	B	3 (4.0)	5 (0.8)	3 (1.1)	3 (100)
Thompson	C1	3 (4.0)	3 (0.5)	3 (1.1)	1 (33.3)
Wien	B	2 (2.7)	13 (2.1)	4 (1.4)	4 (100)
Szentes	I	2 (2.7)	8 (1.3)	4 (1.4)	0 (0)
Oranienburg	C1	2 (2.7)	23 (3.7)	4 (1.4)	1 (25)
London	E1	2 (2.7)	11 (1.8)	4 (1.4)	4 (100)
Havana	G2	1 (1.3)	5 (0.8)	2 (0.7)	2 (100)
Brandenburg	B	1 (1.3)	6 (1.0)	2 (0.7)	2 (100)
Other	—[d]	16 (21.3)	30 (4.8)	26 (9.4)	6 (23.1)
Total		75	625	278	204 (73.4)

[a] Percentage calculated from the total number (75) of infected farms.
[b] Percentage calculated from the total number (625) of infected pigs.
[c] Percentage calculated from the total number (278) of strains analyzed.
[d] Serogroups included B, C1, C2, E1, F, I, K, and M.

Overall, most of the strains showed AR to more than one agent belonging to the same antimicrobial agent family. For instance, strains resistant to cefotaxime were also resistant to ceftiofur (third-generation cephalosporins); those resistant to AMP were also resistant to amoxicillin, likewise for streptomycin and spectinomycin, and tetracycline and doxycycline (Table 2). Accordingly, *Salmonella* strains were classified further by their AR profiles to antimicrobial agent families, according to the OIE ''List of Antimicrobials of Veterinary Importance'' *(10).* Eighty-four percent of the AR strains showed multi-AR patterns (25 different), with 75% showing resistance to three or more antimicrobial agent families (Table 3). The most common multi-AR profiles were aminopenicillins-aminoglycosides-sulfonamides, with 51 strains from 33 farms, seconded by aminopenicillins-phenicols-aminoglycosides-sulfonamides-tetracyclines, with 44 strains from 29 farms (Table 3).

The usual finding was the presence of different multi-AR patterns coexisting within the same farm. In fact, among the 68 farms where two or more *Salmonella* strains were analyzed, only 17.6% presented strains with the same multi-AR patterns.

Results from the questionnaire on antimicrobial agent use in farm animals indicated that 95.7% of the responding farms used medicated feed during the fattening period. Most (58.8%) provided them once, typically during the first month of fattening. The mean number of days under treatment was 18 (95% CI = 16 to 19). Tetracyclines were the agents administered most frequently to fattening pigs (44 farms), then polypeptides (37 farms) and aminopenicillins (22 farms). Most of the farms that administered tetracyclines and aminopenicillins had *Salmonella* strains resistant to these antimicrobial agent families (91% in both cases). However, no statistical relationship was found between the use of a given family of antimicrobial agents and the presence of strains with AR against that family. Only on one farm where colistin was administered was AR against polypeptides found (not data on antibiotic use were available from the other farm that showed one strain with this AR pattern).

Interestingly, the two farms that showed resistance to colistin presented a much higher prevalence (56%) compared with the farms with no resistance to that antimicrobial agent (32.5%), but no statistical significance could be inferred from so few samples.

Risk factor analysis. Questionnaires were received from 79 (98.7%) pig farms, but only 73 (91.2%) were included in the final model because of the presence of missing values. Twelve variables presenting a biologically plausible and significant association with *Salmonella* infection in the univariable analysis were considered. One of them, the number of supplier herds, was excluded from

TABLE 2. *AR of 204* Salmonella *strains isolated from MLN from finishing pigs in northeastern Spain*

Antimicrobial		No. (%) of strains[a]	
Family	Agent	Resistant	With intermediate resistance
Quinolones (1st generation)	Nalidixic acid	34 (16.7)	1 (0.5)
Fluoroquinolones (2nd generation)	Enrofloxacin	0 (0)	0 (0)
	Ciprofloxacin	0 (0)	0 (0)
Cephalosporins (3rd generation)	Cefotaxime	1 (0.5)	1 (0.5)
	Ceftiofur	1 (0.5)	1 (0.5)
Aminopenicillins	Ampicillin	142 (69.6)	0 (0)
	Amoxicillin	143 (70.1)	0 (0)
	Amoxicillin–clavulanic acid	20 (9.8)	30 (14.7)
Phenicols	Chloramphenicol	72 (35.3)	1 (0.5)
Aminoglycosides	Streptomycin	110 (53.9)	37 (18.1)
	Spectinomycin	78 (38.2)	16 (7.8)
	Gentamicin	12 (5.9)	0 (0)
	Neomycin	13 (6.4)	1 (0.5)
Sulfonamides	Sulfisoxazole	155 (76)	1 (0.5)
Pyrimidines	Trimethoprim	61 (29.9)	1 (0.5)
Sulfonamide and pyrimidine	Sulfamethoxazole-trimethoprim	61 (29.9)	0 (0)
Tetracyclines	Tetracycline	172 (84.3)	5 (2.5)
	Doxycycline	149 (73)	19 (9.3)
Polypeptides	Colistin	2 (1)	0 (0)

[a] Categorized as resistant or intermediate, according to CLSI standards *(4)*.

the multivariable model because of the low number of responses obtained ($n = 53$). The final model was constructed with two potential confounders, abattoir (six different slaughterhouses) and province (three), and nine possible explanatory variables: season (autumn, winter, spring, and summer), type of farm (farrow-to-finish farms versus strict finishing units), time of transport from farm to abattoir ($\leq$0.5, $>$0.5, and $\leq$0.75 to $>$0.75 h), percentage of slatted floor ($<$50, 50 to 100, and 100%), box stocking density ($\leq$1.45 versus $>$1.45 pigs per m^2), number of full-time workers ($\leq$1 versus $>$1), rodent control (continuous, sometimes, never), water supply (city water versus own well, rivers, irrigation channels, etc.), and number of different diets during the fattening period (2 versus $>$2). As a significant proportion of farms slaughtered older animals to fulfill the requirements of the Designation of Origin Jamón de Teruel (8 to 9 months of age, and up to 130 kg of live weight), we further included the length of the fattening period ($\leq$5 versus $>$5 months) in the analysis because of its potential association with infection (i.e., longer period of exposure).

Five variables appeared as potential risk factors. Pigs from strict finishing farms, from farms without rodent control programs, from farms with more than one full-time worker, pigs having longer fattening periods ($>$5 months), or pigs that drank water from sources other than the city supply presented a higher probability of being infected (Table 4). In addition, pigs coming from multiple ($>$2) suppliers presented a higher probability of being infected, as compared with those pigs coming from a single supplier (odds ratio [OR] = 4.6; 95% CI = 1.2, 4.5; $P \leq 0.009$), but they were not included in the final analysis because of the low response rate.

DISCUSSION

The present study was a herd-based survey designed to determine both the individual and herd *Salmonella* prevalences in the second most important pig producing region of Spain, and the identification of potential herd-level factors associated with them. A previous survey on pig salmonellosis carried out on pig feces in Spain yielded a herd prevalence of approximately 45% *(24)*. This figure likely underestimated the real prevalence, due to the intermittent shedding of the animals and the low sensitivity of the bacteriological culture, especially when used on pools of less than 20 individual fecal samples (sensitivity of less than 67% *(12)*). Thus, for this study, the expected herd prevalence was estimated to be around 75%, and therefore a sample of 80 herds was considered enough to estimate prevalence, with an acceptable error of $\pm$10%. Likewise, 25 animals per herd were considered sufficient to detect infection if present at levels around 10% *(34)*. Given the number of pigs and herds included, this study was one of the largest carried out in Spain to date.

The use of MLN has some important advantages, as compared with the use of pools of feces. Culturing from MLN improves the sensitivity of the technique because of the absence of competing environmental and intestinal tract saprophytic microorganisms, and/or other substances that could interfere with bacteriological diagnosis. It also reflects more accurately the true infection status of the individual animals *(5)*.

The estimated herd prevalence in finishing pigs was much higher than were those prevalences observed in other studies in Spain, which ranged from 20.3% in Catalonia *(36)* to 43.1% in the entire country *(25)*. The large discrepancy with these results could be because of the

TABLE 3. *Twenty-five multiple AR family patterns found in the 204* Salmonella*-resistant strains isolated from finishing pigs in northeastern Spain*

AR family pattern[a]	No. of strains	Serotype(s)/subspecies involved (no. of strains)
A-C-S-Su-T	44	Typhimurium (31), Rissen (4), 4,[5],12:i:− (3), Wien (2), *arizonae* (1), Kapemba (1), Brandenburg (2)
A-C-S-Su-T-Q1G	15	Typhimurium (13), Goldcoast (2)
A-C-S-Su-T-C3G	1	Bredeney
A-C-S-Su	2	Rissen, Wien
A-C-S-T	2	Goldcoast
A-S-Su-T	51	Typhimurium (19), 4,[5],12:i:− (19), Rissen (9), Thompson (1), Meleagridis (1), Montevideo (1), Derby (1)
A-S-Su-T-Q1G	2	Typhimurium, Kapemba
A-S-Su-T-Px	2	4,[5],12:i:−, Rissen
A-S-Su	6	4,[5],12:i:− (3), *arizonae* (1), Rissen (2)
A-Su-T-Q1G	10	Typhimurium
A-S-T	3	London (2), Rissen (1)
A-S-T-Q1G	2	London
A-S-Q1G	1	Kapemba
C-S-Su-T	5	Typhimurium (2), Rissen (2), Kapemba (1)
C-S-Su	2	Gaminara, Typhimurium
Su-T-Q1G	2	Bredeney, Typhimurium
S-Su-T	3	Derby, Goldcoast, Rissen
S-Su	3	6,7-1,5 (2), Infantis (1)
A-T	1	4,[5],12:i:−
C-T	2	Derby, Havana
S-T	3	Rissen
Su-T	6	Bredeney (1), Derby (4), Reading (1)
T-Q1G	1	Derby
Su-Q1G	1	Enteritidis
Su-C3G	1	Muenster

[a] A, aminopenicillins; C, phenicols; S, aminoglycosides; Su, sulfonamides; T, tetracyclines; Q1G, first-generation quinolones; C3G, third-generation cephalosporins; Px, polypeptides.

different type of sample chosen, i.e., feces, and the smaller number of samples collected per herd *(22)*. Bearing in mind the unknown (but limited) sensitivity of the culture technique used, all the fattening farms analyzed were considered infected. The individual prevalence found in our study was similar to that reported by the EFSA in 2007, using the same matrix and culture technique *(8)*, confirming that *Salmonella* prevalence in finishing pigs in Spain is well above the European average of 10%.

The number of serotypes detected was much larger than the number observed in previous studies in Spain *(25, 36)*. The difference could be related to regional differences, or simply due to a matter of quantity, as the most prevalent *Salmonella* serotypes will usually outnumber the rarer serotypes in pools of feces from groups of *Salmonella*-infected pigs. The relevance of these numerous but ''less important'' serotypes is contingent on the consequences they might have on control programs, based on serology, as most of them belonged to serogroups B, C1, and D1, which might give rise to a significant proportion of positive results *(43)*.

The most prevalent serotypes observed were Typhimurium, its monophasic variant 4,[5],12:i:−, and Rissen, mirroring the results of the 2007 EFSA study *(8)*. While Typhimurium and Rissen have been serotypes frequently isolated, 4,[5],12:i:− is considered an emerging multi-AR associated serotype in Europe, with a clear zoonotic importance requiring surveillance *(26, 28, 40, 44)*.

AR was widespread and similar, as observed in other recent studies in Spain *(1, 24, 35, 36, 41)*. Overall, the antimicrobial agent families affected more by AR were tetracyclines, sulfonamides, and aminopenicillins, as seen in other regions *(27, 30, 36, 42)*. Resistance to the aminopenicillins family was due mostly to the presence of β-lactamases, as the association of amoxicillin and clavulanic acid led to a significant reduction of AR when compared with amoxicillin alone (from 51 to 18%, $P < 0.0001$). In contrast, most of the strains susceptible to the association of sulfonamide and trimethoprim were also susceptible to trimethoprim alone, suggesting that the mechanism of resistance was due largely to the absence of disposable *para*-aminobenzoic acid for bacterial folic acid synthesis.

AR to three or more antimicrobial agent families was predominant among AR strains, with aminopenicillins-aminoglycosides-sulfonamides-tetracyclines and aminopenicillins-phenicols-aminoglycosides-sulfonamides-tetracyclines being the most common. These multi-AR patterns are consistent with those patterns observed previously in Spain *(1, 13, 24, 35, 36, 41)*, confirming its constant and widespread distribution. The diversity of serotypes and multi-AR patterns coexisting within a farm suggests the presence of multiple sources of *Salmonella* infection.

Although the use of antimicrobial agents in pigs could promote the appearance of multiple resistant clones *(20)*, the design of this study prevented proper assessment of the associations between the use of a given antimicrobial agent family and the corresponding AR. Some AR for third-generation cephalosporins (cefotaxime and ceftiofur) were observed, although they are rarely used in swine production (none of the farms sampled used it). *Salmonella* resistance or decreased susceptibility to these drugs has been reported *(13, 24, 36)*, and it is attributed to the existence of mutations in genes encoding extended-spectrum β-lactamases *(39)*. These antimicrobial agents are from the same family as is ceftriaxone, the drug of choice for the treatment of *Salmonella* infection in children *(9)*, which justifies the monitoring of this emerging issue. *Salmonella* AR to polypeptides was found in low frequency as well. Colistin is, however, widely used (>50% of the surveyed farms) for treatment and prevention of enteric gram-negative infections in young pigs during transition to fattening units *(18)*. The high *Salmonella* prevalence observed in pigs from the two farms on which resistance to colistin was detected could be a response to a failure in the antibiotic-based control of this infection at the beginning of the fattening period. Further research on this matter is warranted.

One of the main factors associated with the prevalence of *Salmonella* spp. in finishing pigs was the absence of

TABLE 4. *Variables associated with* Salmonella *prevalence in pigs by multivariable random-effects logistic regression analysis in a study in northeastern Spain*[a]

Variable	Logistic regression parameter				
	B	SE (β)	*P* value	OR[b]	95% CI (OR)
Abattoir[c]					
A[d]				1	
B	1.5	0.7	0.03	4.5	1.1–17.5
C	−0.2	0.7	0.75	0.8	0.2–3.1
D	−2.2	0.8	0.01	0.1	0.02–0.5
E	0.7	0.6	0.20	2.1	0.7–6.4
F	−0.3	0.6	0.62	0.7	0.2–2.3
Province[c]					
Huesca[d]				1	
Teruel	−0.2	0.6	0.70	0.8	0.2–2.7
Zaragoza	0.03	0.6	0.96	1.0	0.3–3.5
Type of farm					
Farrow-to-finish[d]				1	
Finishing	0.9	0.3	0.01	2.5	1.3–4.9
Rodent control					
Continuous[d]				1	
Sometimes	0.4	0.2	0.13	1.4	0.9–2.3
Never	1.5	0.3	0.01	4.3	2.2–8.4
No. of full-time workers					
≤1[d]				1	
>1	0.6	0.2	0.01	1.8	1.1–2.9
Length of the fattening period (mo)					
≤5[d]				1	
>5	1.2	0.4	0.00	3.2	1.6–6.7
Water supply					
City[d]				1	
Other (own wells, rivers, irrigation channels, etc.)	0.6	0.3	0.02	1.9	1.1–3.2
Constant	−2.9	0.4	−6.80	0.0	0.02–0.1

[a] Variance of the herd random effect was 1.02 (standard error was 0.32).
[b] OR, odds ratio.
[c] Variables retained in the model as potential confounders.
[d] Reference category.

rodent control programs. Rodents are considered reservoirs of numerous infection-causing organisms *(29, 31, 32)*. *Salmonella*-infected mice have been detected in pig farms *(15, 33)* and correlated with *Salmonella* infection in other domestic animals *(21)*. As in a previous study *(36)*, the magnitude of the association was large (OR = 4.2), which underscores the role rodents might play in the maintenance of infection in fattening pig farms.

Pigs coming from strict finishing farms were approximately 2.5 times more likely to be *Salmonella* infected, as compared with those from farrow-to-finish farms. The latter might have a lower probability of introducing infection, as these farms usually do not purchase weaners, thus reducing the animal movement and the probability of bringing in subclinically infected pigs *(16, 19)*. In fact, it was observed that pigs coming from a single supplier presented a lower probability of being infected, which supports this hypothesis.

Herd size seems to be positively associated with *Salmonella* prevalence. Larger farms could be reflecting other factors associated with infection, such as the frequency of introduction of new animals, number of suppliers, etc. *(14, 17, 22, 36)*. We did not find such associations, but we found a significant relationship with a variable that could be acting as a surrogate of herd size: the number of full-time workers. The probability of infection was higher when there was more than one full-time worker in the farm (OR = 1.81). In fact, having more than two people present at a finisher site daily has been already associated with elevated *Salmonella* prevalence in U.S. farms *(23)*. In Spain, a larger number of farm workers might relate, for instance, to a higher risk of cross-contamination among premises when strict on-farm hygiene and biosecurity measures are not implemented, something relatively common on Spanish pig farms *(18)*.

Drinking water from sources other than a city supply had been associated previously with higher levels of *Salmonella* seroprevalence in finishing pigs in Spain *(36)*. The need to store water (that may be contaminated) in tanks (that can be contaminated) along with the lack of proper chlorination systems are potential risk factors for *Salmonella* infection on farms *(33)*. In contrast, those farms obtaining water directly from a city supply might not need to store it often (city water is properly chlorinated), thus significantly decreasing the risk of contamination.

Pigs spending more than 5 months in fattening premises were around three times more likely to be *Salmonella* positive. In *Salmonella*-contaminated environments, the longer the exposure period, the higher the probability of becoming infected is. Indeed, sows show a much higher *Salmonella* seroprevalence than do finishing pigs in this region (76.6 versus 35.4%, respectively; data not shown). This observation should be considered when raising heavier pigs for special production such as those pigs from the Designation of Origin Jamón de Teruel.

In conclusion, this study shows that AR *Salmonella* infection is widespread in swine finishing farms. Several factors, associated mostly with hygiene and biosecurity measures, could be largely responsible for the high levels of *Salmonella* prevalence observed in the region. On-farm measures aimed at controlling these factors would not be difficult to implement in the short term, through educating and training farmers. AR might be another factor that could have some influence on *Salmonella* prevalence, but its implication has not been sufficiently studied.

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